iGEM Costa Rica

JUNE- JULY

Laboratory Notebook

June 9

Preparation of Competent Cells (Steps 1-2)

June 13

Preparation of Competent Cells (Steps 3-8)

June 14

Preparation of Competent Cells (Steps 9-12)

Competent cells test

Efficiency: $3,5 \times 10^7$

June 15

Biobrick Resuspension:

Biobrick	Name	Resistance	Plate Position
K1319004	TEV Protease	C	V-90
J23100	Anderson Promoter	A	IV-170

Transformation

Name	Resistance	Result
AraC	C	✓
TetR	C	✓
LacZ	C	✓
LacZ Lambda	A	✗
GFP	A	✓
RBS Elowitz	A	✓
GFP Quencher	C	✓

Anderson Promoter 106	A	✓
TEV Protease	C	✓
Anderson Promoter 100	A	✓
Double T	C	✓
AmilGFP	C	✓
C-	-	✓
C+ (RFPbbC)	C	✓
C+ (RFPbbA)	A	✓

June 16

Biobrick Resuspension:

Biobrick	Name	Resistance	Plate Position
K73174	T7 Terminator	C	I-24K
I712074	T7 Promoter	K	V-6N

Transformation:

Name	Resistance	Result
LacZ Lambda	A	✓
T7 Terminator	C	✗
T7 Promoter	K	✗
C-	-	✓
C+ (RFPbbC)	C	✓
C+ (RFPbbA)	A	✓

Bacterial culture:

Name	Antibiotic
AraC	C
TetR	C
LacZ	C

LacZ Lambda	A
GFP	A
RBS Elowitz	A
GFP Quencher	C
Anderson Promoter 106	A
TEV Protease	C
Anderson Promoter 100	A
Double T	C
AmilGFP	C
LacZ Lambda	A
T7 Terminator	C

June 17

Plasmid isolation:

Miniprep	**Resistance**	**A(260/280)**	**[ng/µL]**
AraC	C	1,77	45,2
TetR	C	1,82	42,6
LacZ	C	1,81	35,6
LacZ Lambda	A		
GFP	A	1,77	45,2
RBS Elowitz	A	1,74	22,2
GFP Quencher	C	1,75	43,8
Anderson Promoter 106	A	1,77	102,2
TEV Protease	C	1,81	60,4
Anderson Promoter 100	A	1,85	137,6
Double T	C	1,80	33,0
AmilGFP	C	1,87	55,3
LacZ Lambda	A	-	-
T7 Terminator	C	-	-

Restriction:

Part	Enzymes
TetR	E+S
LacZ	E+S
AraC	E+S
GFP	E+S
GFP Quencher	E+S
AmilGFP	E+S
TEV Protease	E+S
RBS Elowitz	X+P
Double T	X+P

Ligation:

Upstream part	Downstream part	Backbone
TetR	RBS Elowitz	RFPbbK
LacZ	RBS Elowitz	RFPbbK
AraC	RBS Elowitz	RFPbbK

June 20

Bacterial culture:

Name	Antibiotic
AraC	C
LacZ	C
RBS Elowitz	A
Double T	C
RFPbbC	C
RFPbbA	A

PCR and electrophoresis:

Plasmid isolation products from June 17 and the proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of biobricks.

Preparation of Competent Cells (Steps 1-2).

Biobrick Resuspension:

Biobrick	Name	Resistance	Plate Position
J230101	Anderson Promoter 101	A	IV-17F
J23102	Anderson Promoter 102	A	IV-17H
J23103	Anderson Promoter 103	A	IV-17J
J23104	Anderson Promoter 104	A	IV-17L
J23105	Anderson Promoter 105	A	IV-17N
J23107	Anderson Promoter 107	A	IV-19B
J23108	Anderson Promoter 108	C	IV-4C
J23109	Anderson Promoter 109	C	IV-4E
J23110	Anderson Promoter 110	A	IV-19D
J23111	Anderson Promoter 111	C	IV-4A
J23112	Anderson Promoter 112	A	IV-19F
J23113	Anderson Promoter 113	A	IV-19H
J23114	Anderson Promoter 114	A	IV-19J
J23115	Anderson Promoter 115	A	IV-19L
J23116	Anderson Promoter 116	A	IV-19N
J23117	Anderson Promoter 117	A	IV-19P
J23118	Anderson Promoter 118	A	IV-21B

Ligation:

Upstream part	Downstream part	Backbone
AmilGFP	Double T	RFPbbK
GFP	Double T	RFPbbK
GFP Quencher	Double T	RFPbbK
TEV Protease	Double T	RFPbbK

Transformation:

Name	Antibiotic	Result
T7 promoter	K	✗
Anderson Promoter 101	A	✗
Anderson Promoter 102	A	✓
Anderson Promoter 103	A	✗
Anderson Promoter 104	A	✗
Anderson Promoter 105	A	✓
Anderson Promoter 107	A	✗
Anderson Promoter 108	C	✗
Anderson Promoter 109	C	✗
Anderson Promoter 110	A	✗
Anderson Promoter 111	C	✗
Anderson Promoter 112	A	✗
Anderson Promoter 113	A	✓
Anderson Promoter 114	A	✓
Anderson Promoter 115	A	✓
Anderson Promoter 116	A	✓
Anderson Promoter 117	A	✓
Anderson Promoter 118	A	✗
AmilGFP + Double T	K	✓
GFP + Double T	K	✓
GFP Quencher + Double T	K	✓
TEV protease + Double T	K	✓
C-	-	✓
C+ (RFPbbC)	C	✓
C+ (RFPbbA)	A	✓

PCR and electrophoresis:

A gradient PCR was made in order to optimize annealing temperature for biobrick amplification. The proper controls were made.

Tested temperatures:

- 65°C
- 64°C
- 63°C
- 62°C
- 61°C
- 60°C

Electrophoresis in agarose gel was made to confirm the presence of biobricks. As result we obtained that 62°C is the best annealing temperature.

June 21

Preparation of Competent Cells (steps 3-8).

Plasmid isolation:

Miniprep	**Resistance**	**A(260/280)**	**[ng/µL]**
RFP Coding Device bbA	A	1,85	282,6
RFP Coding Device bbC	C	1,86	250,9
RBS Elowitz	A	1,86	149,2
AraC	C	1,88	138,8
LacZ	C	1,86	195,5
Double T	C	1,87	152,8

Restriction:

Part	**Enzymes**
RFP Coding Device bbA	E+P
RFP Coding Device bbC	E+P
RBS Elowitz	X+P
AraC	E+S
LacZ	E+S

Double T	X+P

Ligation:

Upstream Part	Downstream Part	Backbone
LacZ	RBS Elowitz	RFPbbK
AraC	RBS Elowitz	RFPbbK

June 22

Preparation of Competent Cells (steps 9-12).

Transformation:

Name	**Antibiotic**	**Result**
LacZ+RBS Elowitz	K	✓
AraC+RBS Elowitz	K	✓
Anderson Promoter 101	A	✓
Anderson Promoter 102	A	✓
Anderson Promoter 105	A	✓
Anderson Promoter 107	A	✗
Anderson Promoter 108	C	✓
Anderson Promoter 111	C	✓
Anderson Promoter 112	A	✓
C-	-	✓
C-	K	✗
C+ (RFPbbK)	-	✓
C+ (RFPbbK)	K	✓
C+ (RFPbbC)	-	✓
C+ (RFPbbC)	C	✓

C+ (RFPbbA)	-	✓
C+ (RFPbbA)	A	✓

PCR and electrophoresis:

Plasmid isolation products from June 21 with the proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of biobricks.

Bacterial culture:

Name	**Antibiotic**
LacZ Lambda	A
T7 terminator	C
TetR	C
Anderson Promoter 114	A
Anderson Promoter 105	A
Anderson Promoter 115	A
Anderson Promoter 113	A
Anderson Promoter 116	A
RFPbbK	K
RFPbbT	T

June 23

Plasmid isolation:

Miniprep	**Resistance**	**A(260/280)**	**[ng/µL]**
Anderson Promoter 105	A	1,58	9,1
Anderson Promoter 113	A	1,43	15,6
Anderson Promoter 116	A	1,42	9,2
Anderson Promoter 115	A	1,38	5,7
T7 Terminator	C	1,82	107,5
RFPbbT	T	1,82	32,5
RFPbbK	K	1,84	103,3

TetR	C	1,82	53,5
Anderson Promoter 114	A	1,84	116,6
LacZ Lambda	A	1,66	41,3

The plasmid isolation products with an absorbance below 1,8 (260/280) were discarded.

PCR and electrophoresis:

Plasmid isolation products from June 23 with the proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of biobricks.

Transformation:

Name	**Antibiotic**	**Result**
TEV Protease	C	✓
GFP	A	✗
Anderson Promoter 107	A	✗
GFP + Double T	K	✗
TEV + Double T	K	✗
GFP Quencher + Double T	K	✗
AmilGFP + Double T	K	✗
C+ (RFPbbK)	K	✓
C+ (RFPbbA)	A	✓
C+ (RFPbbC)	C	✓
C-	-	✓
C-	K	✗
C-	A	✗
C-	C	✗

Restriction:

Part	**Enzymes**
T7 Terminator	E+S
TetR	E+S

Anderson Promoter 114	E+S
RBS Elowitz	X+P
RFPbbT	E+P
RFPbbK	E+P

Ligation:

Upstream Part	Downstream Part	Backbone
TetR	RBS Elowitz	RFP bbK
Anterson Promoter 114	RBS Elowitz	RFP bbK

The reactions were incubated under three different treatments:

1. 3 hours at 16°C
2. 10 minutes at 37°C
3. Overnight at 37°C

Bacterial culture:

Name	**Antibiotic**
Anderson Promoter 105	A
Anderson Promoter 113	A
Anderson Promoter 116	A
Anderson Promoter 115	A
LacZ Lambda	A

June 24

Colony PCR:

3 colonies of each ligation with the proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs.

Tested ligations:

- AraC + RBS Elowitz + RFPbbK
- LacZ + RBS Elowitz + RFPbbK

As result we obtained that colonies 1 and 2 had the ligation AraC + RBS Elowitz + RFPbbK, and colonies 1 and 3 had LacZ + RBS Elowitz + RFPbbK.

Transformation:

Name	Antibiotic	Result
TetR+RBS Elowitz (1)	K	✓
Anterson Promoter 114 + RBS Elowitz (1)	K	✗
TetR+RBS Elowitz (2)	K	✗
Anterson Promoter 114 + RBS Elowitz (2)	K	✗
TetR+RBS Elowitz (3)	K	✓
Anterson Promoter 114 + RBS Elowitz (3)	K	✗
GFP	A	✓
Anderson Promoter 107	A	✗
C+ (RFPbbK)	K	✓
C+ (RFPbbA)	A	✓
C-	-	✓
C-	K	✗
C-	A	✗

*Numbers represent the different treatments during ligation:

1. 3 hours at 16°C
2. 10 minutes at 37°C
3. Overnight at 37°C

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]
Anderson Promoter 105	A	1,81	13,9
Anderson Promoter 113	A	1,60	8,3
Anderson Promoter 115	A	1,66	12,4
Anderson Promoter 116	A	1,99	0,7
LacZ Lambda	A	2,01	56,3

*There´s something wrong with Plasmid Isolation. All plasmid isolation products were discarded.

Bacterial culture:

Name	**Antibiotic**
Anderson Promoter 101	A
Anderson Promoter 102	A
Anderson Promoter 105	A
Anderson Promoter 105	A
Anderson Promoter 108	C
Anderson Promoter 111	C
Anderson Promoter 112	A
Anderson Promoter 113	A
Anderson Promoter 116	A
Anderson Promoter 115	A
LacZ Lambda	A
AraC + RBS Elowitz (C1)	K
AraC + RBS Elowitz (C2)	K
LacZ + RBS Elowitz (C1)	K
LacZ + RBS Elowitz (C3)	K

*C# represents the colony used to prepare the liquid culture, in this case we used the colonies that amplified in the colony PCR.

Ligation:

Upstream Part	Downstream Part	Backbone
GFP	**Double T**	**RFPbbK**
AmilGFP	**Double T**	**RFPbbK**
GFP Quencher	**Double T**	**RFPbbK**
TEV Protease	**Double T**	**RFPbbK**

June 25

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]
Anderson Promoter 101	A	1,86	234,1
Anderson Promoter 102	A	1,86	352,9
Anderson Promoter 105	A	2,13	17,7
Anderson Promoter 105	A	1,87	150,6
Anderson Promoter 108	C	2,19	7,5
Anderson Promoter 111	C	2,18	2,6
Anderson Promoter 112	A	2,10	9,8
Anderson Promoter 113	A	1,99	6,0
Anderson Promoter 115	A	1,87	8,7
Anderson Promoter 116	A	2,33	7,5
LacZ Lambda	A	1,87	78,1
AraC + RBS Elowitz (C1)	K	1,86	155,0
AraC + RBS Elowitz (C2)	K	1,86	204,0
LacZ + RBS Elowitz (C1)	K	1,86	317,6
LacZ + RBS Elowitz (C3)	K	1,86	339,3

*We still have problems with minipreps. We noticed that since the lysis step some reactions had different behavior than usual.

Plasmid isolation products with an absorbance below 1,8 (260/280) or over 1,99 (260/280) were discarded.

PCR and electrophoresis:

Plasmid isolation products from June 25 with proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of biobricks and ligations.

June 26

We discovered why the plasmid isolation is having low yield: some of our cultures in Petri dishes were contaminated with yeast.

Transformation:

Name	Antibiotic	Result
T7 Promoter	K	✗
Anderson Promoter 102	A	✓
Anderson Promoter 105	A	✓
Anderson Promoter 111	C	✗
Anderson Promoter 112	A	✓
Anderson Promoter 113	A	✓
Anderson Promoter 115	A	✓
Anderson Promoter 116	A	✓
Anderson Promoter 117	A	✓
Anderson Promoter118	A	✓
C+ (RFPbbK)	K	✓
C+ (RFPbbA)	A	✓
C+ (RFPbbC)	C	✓
C-	-	✓
C-	K	✗
C-	A	✗
C-	C	✗

Colony PCR:

Two colonies (C1 and C2) from the ligation Anderson promoter 114 + RBS Elowitz and the proper control were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs.

Bacterial culture:

Name	Antibiotic
GFP	A
TEV Protease	C
Anderson Promoter 107	A

Preparation of competent cells (Steps 1-2).

June 27

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]
GFP	A	1,82	101,2
TEV Protease	C	1,76	232,2
Anderson Promoter 107	A	1,68	153,2

Two plasmid isolation products had an absorbance below 1,8 (260/280). They were not discarded since the concentration of plasmid was over the optimum.

PCR and eletrophoresis:

The last plasmid isolation products with proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs.

Bacterial culture:

Name	Antibiotic
T7 Promoter	K
Anderson Promoter 111	C

Preparation of competent cells (Steps 3-8).

Ligation:

Upstream Part	Downstream Part	Backbone
Anderson Promoter 114	**RBS Elowitz**	**RFPbbk**

The reactions were incubated under three different treatments:

1. 3 hours at 16°C
2. 10 minutes at 37°C
3. Overnight at 37°C

*Volumes added:

- 4µL of Promoter
- 4µL of RBS
- 2µL of Backbone

Transformation:

Name	**Antibiotic**	**Result**
Anderson Promoter 108	C	✗
Anderson Promoter 111	C	✗
RFPbbC	C	✓
RFPbbK	K	✓
GFP + DT + RFPbbK	K	✓
AGFP + DT + RFPbbK	K	✓
QGFP + DT + RFPbbK	K	✗
TEV + DT + RFPbbK	K	✓
C-	-	✓
C-	K	✗
C-	C	✗
C-	-	✓

Preparation of Competent Cells (steps 3-8).

June 28

33

The constructs with inducible promoters and RBS were prepared for sequencing.

Preparation of Competent Cells (steps 9-12).

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]
Anderson Promoter 105	A	1,74	168,1
Anderson Promoter 102	A	1,83	242,9
Anderson Promoter 112	A	1,85	179,9
Anderson Promoter 113	A	1,85	171,0
Anderson Promoter 115	A	1,84	223,2
Anderson Promoter 116	A	1,85	246,9
Anderson Promoter 117	A	1,84	291,2
Anderson Promoter 118	A	1,85	120,1

PCR and eletrophoresis:

Plasmid isolation products from the last experiment with proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of biobricks. It was confirmed that the DNA was correct in all the samples.

Restriction:

Part	Enzymes
Anderson Promoter 100	E+S
Anderson Promoter 106	E+S
Anderson Promoter 115	E+S
RFPbbK (Backbone)	E+P

Ligation:

Upstream Part	Downstream Part	Backbone
Anderson Promoter 100	**RBS Elowitz**	**RFPbbK**
Anderson Promoter 106	**RBS Elowitz**	**RFPbbK**
Anderson Promoter 115	**RBS Elowitz**	**RFPbbK**

Transformation:

Name	**Antibiotic**	**Result**
10X His Tag	C	✗
Anderson Promoter + RBS Elowitz (1)	K	✗
Anderson Promoter + RBS Elowitz (2)	K	✗
RFPbbC	C	✓
RFPbbK	K	✓
C-	C	✗
C-	K	✗
C-	-	✓

June 29

Colony PCR and electrophoresis:

Colonies from each of the next ligations and the proper control were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs. It was confirmed that the ligation was not correct in any of the analized colonies.

- ●GFP + Double T (C2)
- ●GFP + Double T (C3)
- ●TEV Protease + Double T (C2)
- ●AmilGFP + Double T (C1)
- ●AmilGFP + Double T (C2)
- ●AmilGFP + Double T (C3)

*C# refers to the colony selected for PCR amplification.

Transformation:

Name	Antibiotic	Result
10-His Tag (1)	C	✗
10-His Tag (2)	C	✓
Anderson Promoter 114 + RBS Elowitz (1)*	K	✓
Anderson Promoter 114 + RBS Elowitz (1)**	K	✗
Anderson Promoter 114 + RBS Elowitz (2)*	K	✓
Anderson Promoter 114 + RBS Elowitz (2)**	K	✗
Anderson Promoter 114 + RBS Elowitz (3)*	K	✗
Anderson Promoter 114 + RBS Elowitz (3)**	K	✗
Anderson Promoter 100 + RBS Elowitz *	K	✓
Anderson Promoter 100 + RBS Elowitz **	K	✓
Anderson Promoter 106 + RBS Elowitz *	K	✓
Anderson Promoter 106 + RBS Elowitz **	K	✓
Anderson Promoter 115 + RBS Elowitz *	K	✓
Anderson Promoter 115 + RBS Elowitz **	K	✓
C+ (RFPbbK)*	K	✓
C+ (RFPbbK)**	K	✓
C+ (RFPbbC)*	C	✓
C+ (RFPbbC)**	C	✓
C-*	-	✓
C-**	-	✓

In this experiment we tested two lots of competent cells: lot 1 (*) and lot 2 (**)

The reactions were incubated under three different treatments:

1. 3 hours at 16°C
2. 10 minutes at 37°C
3. Overnight at 37°C

ARN isolation:

Culture	OD (600)	A (260/280)	[ng/µL]
DH5α (1)	0,876	2,04	110,1
DH5α (2)	0,553	2,12	114,2
DH5α (3)	0,415	2,13	142,1

The bacterial cultures were incubated under three different treatments:

1. Overnight
2. 4 hours
3. 2 hours

Restriction:

Part	Enzymes
LacZ + lambda	E+S
TetR	E+S

Ligation:

Upstream Part	Downstream Part	Backbone
GFP	**Double T**	**RFPbbT**
GFP Quencher	**Double T**	**RFPbbT**
AmilGFP	**Double T**	**RFPbbT**
GFP	**T7 Terminator**	**RFPbbT**
LacZ + lambda	**RBS Elowitz**	**RFPbbT**
TetR	**RBS Elowitz**	**RFPbbT**

June 29

Restriction:

Backbone	Enzymes
RFPbbA	E+P
RFPbbC	E+P
RFPbbK	E+P

RFPbbT	E+P

Retrotranscription and electrophoresis:

A retrotranscription from the products of ARN isolation from DH5α (June 29) was made. Once obtained the cDNA it was quantified.

Treatment	**A (260/280)**	**[ng/µL]**
1	1,43	645,1
2	1,42	446,7
3	1,43	544,9

The last cDNA was tested by electrophoresis in agarose gel in which a positive result was obtained.

Transformation:

Name	**Antibiotic**	**Result**
TetR + RBS	K	✓
(LacZ + Lamda) + RBS	K	✓
C+ (RFPbbK)	K	✓
C-	-	✓

Bacterial culture:

Name	**Antibiotic**
10X His tag	C

Colony PCR and electrophoresis:

Colonies from each of the next ligations and the proper control were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs. The correct ligation was confirmed.

- Anderson Promoter 100 + RBS
- Anderson Promoter 106 + RBS
- Anderson Promoter 115 + RBS

July 18

Restriction and electrophoresis

Backbone	**Enzymes**
And. Prom. 101	E + S
And. Prom. 106	E + S
And. Prom. 117	E + S
TEV + HT + DT	X + P

The Agarose (2%) Gel Electrophoresis confirmed the digestion of **And. Prom. 101** and **And. Prom. 106**.

Ligation:

Upstream Part	Downstream Part	Backbone
gB2	**-**	**RFPbbK**
gB3	**-**	**RFPbbK**
gB4	**-**	**RFPbbK**
gB6	**-**	**RFPbbK**
And. Prom. 101	**RBS Elowitz**	**RFPbbK**
And. Prom. 106	**RBS Elowitz**	**RFPbbK**
LacZ + RBS	**TEV + 10HT + DT**	**RFPbbC**

Transformation:

Name	**Antibiotic**	**Result**
Interlab Negative Control	C	X
Interlab Positive Control	C	X

And. Prom. 101 + RBS Elowitz	K	X
And. Prom. 106 + RBS Elowitz	K	X
gB2 (1)	K	X
gB2 (2)	K	X
gB3 (1)	K	X
gB3 (2)	K	X
gB4 (1)	K	X
gB4 (2)	K	X
gB6 (1)	K	X
gB6 (2)	K	X
C+ K	K	X
C+ C	C	X
C-	-	X

Bacterial culture:

Name	**Antibiotic**
Interlab D1	C
Interlab D2	C
Interlab D3	C
Interlab C+	C
Interlab C-	C
And. prom. 117	A

July 19

Ligation:

Upstream Part	Downstream Part	Backbone
gB2	**-**	**RFPbbK**

gB3	**-**	**RFPbbK**
gB4	**-**	**RFPbbK**
gB6	**-**	**RFPbbK**
And. Prom. 101	**RBS Elowitz**	**RFPbbK**
And. Prom. 106	**RBS Elowitz**	**RFPbbK**
LacZ + RBS	**TEV + 10HT + DT**	**RFPbbC**

Transformation

The transformation of July 18 was repeated using the totality of the ligation.

Name	**Antibiotic**	**Result**
Interlab Negative Control	C	✓
Interlab Positive Control	C	✓
And. Prom. 101 + RBS Elowitz	K	✓
And. Prom. 106 + RBS Elowitz	K	✓
gB2 (1)	K	✓
gB2 (2)	K	✓
gB3 (1)	K	✓
gB3 (2)	K	✓
gB4 (1)	K	✓
gB4 (2)	K	✓
gB6 (1)	K	✓
gB6 (2)	K	✓
C+ K	K	✓
C+ C	C	✓
C-	-	X

July 20

Transformation

The transformation of July 18 was repeated using the totality of the ligation.

Name	Antibiotic	Result
Interlab Negative Control	C	✓
Interlab Positive Control	C	✓
And. Prom. 101 + RBS Elowitz	K	✓
And. Prom. 106 + RBS Elowitz	K	✓
gB2 (1)	K	✓
gB2 (2)	K	X
gB3 (1)	K	✓
gB3 (2)	K	X
gB4 (1)	K	✓
gB4 (2)	K	X
gB6 (1)	K	✓
gB6 (2)	K	X
C+ K	K	✓
C+ C	C	✓
C-	-	X

Restriction and electrophoresis

Backbone	Enzymes
Prom. T700 + RBS	E +P
Prom. T704 + RBS	E + P
Prom. T707 + RBS	E + P
Prom. And. 117	E + S

Ligation:

Upstream Part	Downstream Part	Backbone
Prom. T700 + RBS Elowitz	**-**	**RFPbbC**
Prom. T704 + RBS Elowitz	**-**	**RFPbbC**
Prom. T707 + RBS Elowitz	**-**	**RFPbbC**

July 21

Colony PCR and electrophoresis

The colonies obtained from the transformation were amplified from the indexed plates previously prepared. The cPCR confirmed the assembly of:

- gB2
- gB3
- gB5
- And. Prom. J23101 + RBS
- And. Prom. J23106 + RBS

Ligation

Upstream Part	Downstream Part	Backbone
TEV - 10HT	**TT7**	**RFPbbA**
GFP	**10 His Tag**	**RFPbbK**

Transformation

Name	**Antibiotic**	**Result**
gB2	K	X
gB3	K	X
gB4	K	X
gB6	K	X
GFP + 10HT	K	✓

TEV - 10HT + TT7	A	✓
PT700 + RBS	C	X
PT704 + RBS	C	X
PT707 + RBS	C	X
PA17 + RBS	C	X
PI2 - RBS + TEV - 10HT -DT	C	✓
C+ K	K	✓
C+ C	C	✓
C-	-	✓

July 22

Plasmid isolation:

Miniprep	**Resistance**	**A(260/280)**	**[ng/µL]**
And. Prom. J23106 + RBS (1)	K	1,86	151,1
And. Prom. J23106 + RBS (2)	K	1,86	167.9
And. Prom. J23101 + RBS (1)	K	1,87	169,8
And. Prom. J23101 + RBS (2)	K	1,87	165,8
gB3	K	1,87	178,8
gB6	K	1,88	168,4

Restriction

Backbone	**Enzymes**
GFP + DT	X + P
AGFP + DT	X + P
Prom. And. J23101 + RBS	E + S
Prom. And. J23106 + RBS	E + S
RFPbbA	X + P

gB2	X + P

PCR and Electrophoresis

Plasmid isolation products from the last experiment with proper controls were tested by PCR. The electrophoresis confirmed the following pieces:

- Prom. And. J23106 + RBS
- Prom. And. J23101 + RBS

Ligation

Upstream Part	Downstream Part	Backbone
Prom. And. J23106 + RBS	**AmilGFP + DT**	**RFPbbA**
Prom. And. J23106 + RBS	**GFP + DT**	**RFPbbA**
Prom. And. J23106 + RBS	**gB6**	**RFPbbA**
Prom. LacZ	**gB3**	**RFPbbA**

Competent Cell Preparation

BL21 and DH5α *E. coli* cultures were prepared.

Colony PCR and electrophoresis

The colonies obtained from the transformation were amplified directly. The cPCR confirmed the assembly of:

- TEV - 10HT + TT7

GFP + 10HT was shown not to be correctly ligated.

July 23rd

Team members in the laboratory: Sofía and Rafael

Electrophoresis:

The Agarose (2%) Gel Electrophoresis confirmed the digestion of all the constructs, made on July 21st.

Restriction:

Part	Enzymes
TEV Protease + 10HT + T7 Terminator	X+P

Ligation:

Upstream Part	Downstream Part	Backbone
gB2	**-**	**RFPbbA**
gB3	**-**	**RFPbbA**
gB4	**-**	**RFPbbA**
gB6	**-**	**RFPbbA**
And. Prom. 101 + RBS Elowitz	**AmilGFP + Double T**	**RFPbbA**
And. Prom. 106 + RBS Elowitz	**AmilGFP + Double T**	**RFPbbA**
And. Prom. 101 + RBS Elowitz	**GFP + Double T**	**RFPbbA**
And. Prom. 106 + RBS Elowitz	**GFP + Double T**	**RFPbbA**
TEV Protease + 10HT	**T7 Terminator**	**RFPbbA**
GFP	**10HT**	**RFPbbK**

July 24th

Team members in the laboratory: Rafael

Transformation

Name	Resistance	Result
gB1	A	✓
gB2	A	✓

gB3	A	✓
gB4	A	✗
gB5	A	✓
gB6	A	✓
And. Prom. 106 + RBS Elowitz + GFP + DT	A	✓
And. Prom. 101 + RBS Elowitz + GFP + DT	A	✓
GFP + 10HT	K	✓
Prom. T70 + RBS	C	✓
Prom. T74 + RBS	C	✓
Prom. T77 + RBS	C	✓
And. Prom. 117 + RBS Elowitz	C	✓
LacZ + RBS Elowitz + TEV Protease + DT (1)	C	✗
LacZ + RBS Elowitz + TEV Protease + DT (2)	C	✗
LacZ + RBS Elowitz + TEV Protease + DT (3)	C	✗
LacZ + RBS Elowitz + TEV Protease + DT (4)	C	✓
C+ (RFPbbA)	A	✓
C+ (RFPbbC)	C	✓
C+ (RFPbbK)	K	✓
C-	A	✓
C-	C	✓
C-	K	✓
C-	-	✓

July 25th

Team members in the laboratory: Rafael and Sofía

Colony PCR and electrophoresis:

6 colonies of each ligation with the proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs.

Tested ligations:

Name	C#1	C#2	C#3	C#4	C#5	C#6
And. Prom. 117 + RBS Elowitz + RFPbbC	✓	✗	✓	✗	✗	✗
Prom. T70 + RBS Elowitz + RFPbbC	✓	✗	✓	✗	✗	✗
Prom. T74 + RBS Elowitz + RFPbbC	✓	✓	✗	✗	✗	✗
Prom. T77 + RBS Elowitz + RFPbbC	✓	✗	✓	✗	✗	✗
GFP + 10HT + RFPbbK	✗	✗	✗	✗	✓	✗
And. Prom. 101 + RBS Elowitz + GFP + DT + RFPbbA	✗	✗	✓	✓	✗	✗
And. Prom. 106 + RBS Elowitz + GFP + DT + RFPbbA	✓	✗	✓	✗	✗	✗
LacZ + RBS Elowitz + TEV Protease + DT + RFPbbC	✗	✗	✓	✓	✗	✗

*C# refers to the colony selected for PCR amplification.

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]

TEV Protease + 10HT + TT7 Terminator	A	1,85	322,2

Restriction:

Part	Enzymes
TEV Protease + 10HT + T7 Terminator	X+P
gB1	X+P
gB5	X+P

July 26th

Team members in the laboratory: Paula, Samantha and Sofía

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]
And. Prom. 101 + RBS Elowitz + GFP + DT (C#3)	A	1,86	99,3
And. Prom. 101 + RBS Elowitz + GFP + DT (C#4)	A	1,86	238,0
And. Prom. 106 + RBS Elowitz + GFP + DT (C#1)	A	1,85	287,5
And. Prom. 106 + RBS Elowitz + GFP + DT (C#3)	A	1,83	128,9
And. Prom. 117 + RBS Elowitz (C#1)	C	1,84	115,2
And. Prom. 117 + RBS Elowitz (C#3)	C	1,88	78,2
GFP + 10HT (C#5)	K	1,86	141,5
Prom. T70 + RBS Elowitz (C#1)	C	1,82	102,6
Prom. T70 + RBS Elowitz	C	1,85	77,5

(C#3)			
Prom. T74 + RBS Elowitz (C#1)	C	1,83	100,0
Prom. T74 + RBS Elowitz (C#2)	C	1,86	68,5
Prom. T77 + RBS Elowitz (C#1)	C	1,86	102,9
Prom. T77 + RBS Elowitz (C#3)	C	1,79	77,2
LacZ + RBS Elowitz + TEV Protease + DT (C#4)	C	1,86	277,6
LacZ + RBS Elowitz + TEV Protease + DT (C#5)	C	1,87	139,2

*C# represents the colony used to prepare the liquid culture, in this case we used the colonies that amplified in the colony PCR.

PCR and electrophoresis:

Plasmid isolation products from July 26th with proper controls were tested by PCR, and electrophoresis in agarose gel was made, the next ligations were confirmed:

- And. Prom. 117 + RBS Elowitz (C#1)
- And. Prom. 117 + RBS Elowitz (C#3)
- Prom. T70 + RBS Elowitz (C#1)
- Prom. T70 + RBS Elowitz (C#3)
- Prom. T74 + RBS Elowitz (C#1)
- Prom. T74 + RBS Elowitz (C#2)
- Prom. T77 + RBS Elowitz (C#1)
- Prom. T77 + RBS Elowitz (C#3)
- LacZ + RBS Elowitz + TEV Protease + DT (C#4)
- LacZ + RBS Elowitz + TEV Protease + DT (C#5)

July 27th

Team members in the laboratory: Samantha

Restriction:

Part	Enzymes
And. Prom. 101 + RBS Elowitz	E + S
And. Prom. 106 + RBS Elowitz	E + S
And. Prom. 117 + RBS Elowitz	E + S
Prom. T70 + RBS Elowitz	E + S
Prom. T74 + RBS Elowitz	E + S
Prom. T77 + RBS Elowitz	E + S
GFP + DT	X + P
10HT	X + P

July 28th

Team members in the laboratory: Sofía, Pablo, Rafael and Samantha

Ligation:

Upstream Part	Downstream Part	Backbone
Prom. T70 + RBS Elowitz	**TEV Protease + 10HT + T7 Terminator**	**RFPbbK**
Prom. T74 + RBS Elowitz	**TEV Protease + 10HT + T7 Terminator**	**RFPbbK**
Prom. T77 + RBS Elowitz	**TEV Protease + 10HT + T7 Terminator**	**RFPbbK**
GFP	**10HT**	**RFPbbK**
gB1	**-**	**RFPbbA**
gB2	**-**	**RFPbbA**
gB4	**-**	**RFPbbA**
gB5	**-**	**RFPbbA**

Colony PCR and electrophoresis:

5 colonies of each gBlock, from July 24th, with the proper controls were tested by PCR, and electrophoresis in agarose gel was made to confirm the presence of the constructs.

Tested ligations:

Name	C#1	C#2	C#3	C#4	C#5	C#6
gB1 + RFPbbA	✗	✗	✗	✗	✗	✗
gB2 + RFPbbA	✗	✗	✗	✗	✗	✗
gB3 + RFPbbA	✓	✗	✗	✗	✗	✗
gB4 + RFPbbA	✓	✓	✓	✗	✗	✗
gB5 + RFPbbA	✗	✗	✗	✗	✗	✗
gB6 + RFPbbA	✗	✗	✗	✗	✗	✗

*C# refers to the colony selected for PCR amplification.

July 29th

Team members in the laboratory: Sofía, Rafael and Paula

Plasmid isolation:

Miniprep	Resistance	A(260/280)	[ng/µL]
gB3 (C#1)	A	1,86	86,05
gB4 (C#1)	A	1,85	131,5
gB4 (C#2)	A	1,85	141,5
gB4 (C#3)	A	1,84	126,7

*C# represents the colony used to prepare the liquid culture, in this case we used the colonies that amplified in the colony PCR.

PCR and electrophoresis:

Plasmid isolation products from July 29th and the proper controls were tested by PCR, and electrophoresis in agarose gel was made, the next gBlocks were confirmed:

- gB4 C#1
- gB4 C#2

Restriction:

Part	Enzymes
gB4 C#1	X + P
gB4 C#2	X + P
10HT	X + P
TEV Protease + 10HT + T7 Terminator	X + P
GFP	E + S
Prom. T70 + RBS Elowitz	E + S
Prom. T74 + RBS Elowitz	E + S
Prom. T77 + RBS Elowitz	E + S
RFPbbA	E + P